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Can Serum IgG Antiganglioside Antibodies to GM1, GM3 and GD1a be Used as Markers in Patients with Ischemic Stroke?

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Our aim is to verify whether serum IgG antiganglioside antibodies (anti-GM1, anti-GD1a and anti-GM3) can be used as markers for patients with ischemic stroke. We showed the results of the antibody titer obtained by ELISA technique. There were 21 sera from patients under 75 years of age with ischemic strokes received between 2017-2020 from University First MHAT – Sofia, "St. John Krastitel". Three patients had a very weak indication for demyelination. Five had indication that correlates with possible metabolic abnormalities. The conclusion that could be drawn was that the antiganglioside antibodies titer was not decisive and could not be used as biomarker in stroke patients, and did not directly correlate with stroke severity, size of the ischemic area (reported by computed tomography data), and likely recovery prognosis. In ischemic strokes none of the patients had an indication of neuronal damage, as in the case with severe attacks of multiple sclerosis or Alzheimer's disease.

Key words: serum antiganglioside antibodies, biomarkers, ischemic stroke, ELISA

Introduction

The expression patterns of gangliosides vary in different tissues, during different life periods, as well as in various pathologies [1, 9, 11, 12]. The antiganglioside antibodies (AGAs) have the potential to be suitable diagnostic and therapeutic biomarkers for many brain abnormalities [14]. The ganglioside composition is related with a certain disorder [14]. Our team has been working with multiple sclerosis (MS), Alzheimer's disease (AD) and aging people for many years [7, 8, 16].

The aim of the present study was to examine whether serum IgG antiganglioside (anti-GM1, anti-GD1a and anti-GM3) antibodies could be used as markers for patients with ischemic stroke. To investigate whether and to what extent higher titers of these

antiganglioside antibodies correlate with stroke severity, ischemic area size (reported by computer tomography data), and whether they are associated with reversal of symptoms.

Materials and Methods

We had 21 sera from patients under 75 years of age with ischemic strokes received from the University First MHAT – Sofia "St. John Krastitel" from 2017 to 2020. All sera were taken before any therapy or immune intervention and they were analyzed in series. All participants agreed to be included in research studies and signed informed consent according to the Declaration of Helsinki. Statistical assay by Student's t-test using statistical package was performed. The results were reported as mean values ± SEM (Standard Error of the Mean) of three independent experiments. Differences were regarded as significant at $p<0.05$. In our work we applied our modification on enzyme-linked immunosorbent assay (ELISA technique). The optical density was detected by ELISA reader Sunrise. The experiments were in triplicate [7, 8].

Results and Discussion

Here we present data from analysis of IgG antibodies against GM1, GD1a and GM3 in patients with stroke (**Table 1**).

Three patients had high titer of anti-GM1 antibodies (a weak indication for demyelination). Five patients showed increased levels of anti-GM3 antibodies-indication that is related with possible metabolic abnormalities (for example diabetes) and/or with possible problems with endothelium. None of the patients had any elevations of anti-GD1a antibodies, which is indicative for neuronal damage, such as severe MS attack and/or AD. Interestingly, in three of the patients, an increased titer of two of the antibodies was observed at the same time, and this demonstrates the dynamic of the gangliosides ratio. The results show that neurons were not damaged enough to be detected by the titer of antibodies to GD1a, as it is in the case of severe attack of MS and in AD. These data suggest that antibodies titers did not directly correlate with stroke severity. From our previous research, it has been concluded that there is a correlation between brain and serum antibodies in various neurological diseases (such as MS) due to disruption of the integrity of the blood-brain barrier (BBB) [7, 8, 16].

There are data showing that anti-GT1b and anti-GM1 (IgM and IgG) antibodies can transiently increase after stroke, but their titers are not associated with late post-apoplectic epilepsy [5]. On the other hand, an elevated titer of anti-GD1b antibodies was observed in patient diagnosed with acute motor axonal neuropathy [2]. Anti-GQ1b ganglioside antibodies are a serological marker of the Miller Fisher syndrome (MFS), a variant of Guillian-Barre syndrome (GBS) and are believed to be the principal pathogenic mediators of the disease [4, 6].

Diseases as epilepsy, Parkinson's disease, Huntington's disease, and melanoma may include impaired ganglioside metabolism [8, 9, 12]. The accumulation of GM2 and GM3 ganglioside types has been proposed as indicative of a mechanism of interactions between stroke, AD and other neurodegenerative diseases and disorders *in vitro* and in rat

Table 1. Titers of anti-GD1a, anti-GM1 and anti-GM3 antibodies in sera from stroke patients

Stroke patients			Anti GD1a antibodies titers	Anti GM1 antibodies titers	Anti GM3 antibodies titers
A	66 years old	female	-	+ 1:40	+ 1:200
B	70 years old	female	-	-	-
C	52 years old	male	-	-	-
D	72 years old	female	-	-	-
E	70 years old	female	-	-	-
F	71 years old	male	-	+ 1:40	+ 1:100
G	70 years old	female	-	-	+ 1:200
H	71 years old	female	-	-	-
I	75 years old	female	-	-	-
J	75 years old	female	-	-	-
K	71 years old	female	-	-	-
L	64 years old	male	-	-	-
M	72 years old	male	-	-	-
N	66 years old	female	-	-	-
O	70 years old	female	-	-	-
P	52 years old	male	-	-	-
Q	72 years old	female	-	-	-
R	70 years old	female	-	-	-
S	71 years old	male	-	-	+ 1:100
T	70 years old	female	-	-	-
U	71 years old	female	-	+ 1:40	+ 1:400

models [1]. The protective role of GM1 ganglioside against brain hypoxia-ischemia was established in animal model [13] and the increased levels of gangliosides in damaged cortex have proposed protection against ischemic damage [10, 15].

In an animal model of MS, we have shown high content of GD1a and increased titer of antibodies against this ganglioside in a blood test, just before the onset of signs of the disease. Thus, serum GD1a may be suggested as a biomarker of axonal BBB disruption, which provides an impetus to initiate early therapy [16]. Therefore, if screening is done and early therapy is started, the development of the disease can be prevented.

AGAs can target immune attack against neuronal cells and to neutralize their complement inhibitory activity. AGAs are important especially in acquired demyelinating immune-mediate neuropathies, like MS, GBS and its variant, the MFS [3, 6].

Conclusion

The conclusion is that the titer of antiganglioside antibodies to GM1, GM3 and GD1a is not decisive and cannot be used as biomarker in ischemic stroke patients. Higher titers of these anti-ganglioside antibodies did not directly correlate with stroke severity, size of the ischemic area (reported by computed tomography data), and likely recovery prognosis.

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